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The Vitamin-B_{12} Activity of Mullet and Shark Serum

THEODORE J. STARR, DOMINGO MARTINEZ, AND WALTER FOSBERG

Gulf Fishery Investigations, U. S. Fish and Wildlife Service, Galveston, Texas

ABSTRACT

Microbiological assay procedures using *Euglena gracilis*, *z* strain, were employed to measure the vitamin-B_{12} activity of samples of mullet, shark, and human sera. The average activity was 10.7, 0.84, and 1.15 mμg/ml, respectively. The relatively higher activity of mullet sera compared to shark sera may reflect significant physiological differences between these subclasses of fish and possibly different feeding habits.

Bacteria isolated from mullet intestinal contents were grown in a vitamin B_{12}-deficient medium. Of 8 cultures tested, 6 had vitamin-B_{12} activity when assayed with *Escherichia coli* 113-3 and 1 had activity for *Euglena*. In other experiments concentrates of cell residues were assayed. Two out of 4 supposedly negative vitamin-B_{12} producers had activity for *Euglena*.

INTRODUCTION

The economy of the oceans is influenced to a great extent by the diverse physiological activities of its microscopic communities. Many of these microscopic populations are dependent upon the availability of specific nutrilites or essential growth factors for growth and development. Of those marine microorganisms that have been studied in the laboratory, several diatoms (Hutner and Provasoli 1953, and Droop 1955) and algal flagellates (see list compiled in Provasoli and Pintner 1953) have a requirement for some member of the vitamin-B_{12} family of compounds.

Vitamin B_{12}-active substances are added to the environment as a result of the metabolic activities of various marine microorganisms. Ericson and Lewis (1953) found that 70 per cent of 34 bacterial cultures isolated from marine sources produced vitamin B_{12}-active compounds. Similar data were obtained by Starr *et al.* (1957), although different assay procedures were used. Water movement and other physical factors undoubtedly aid in the dissemination of these products of microbial origin. Thus, these products may influence distant as well as neighboring populations. The suspended matter of sea water with its inherent and/or adhering nutrients contained appreciable quantities of vitamin B_{12} (Starr 1956, and Burkholder and Burkholder 1956). Ericson and Lewis (1953), who assayed the vitamin-B_{12} activity of seaweed, attributed activity to synthesis by bacteria living epiphytically on the algae rather than to the synthesis by the alga itself.

Several investigatiors have reported the presence of vitamin B_{12}-active substances in marine vertebrates and invertebrates. Masaaki (1952) measured and compared the vitamin-B_{12} activity of the organs of fish. He reported also on the presence of vitamin B_{12} in univalves and bivalves (Masaaki 1953). Maxwell (1952) found that molluscs are, in general, richer in vitamin B_{12} than echinoderms, crustaceans, or annelids. Oysters, clams, and to a lesser extent shrimp had appreciable vitamin-B_{12} activity (Robbins *et al.* 1951) suggesting that algal flagellates, an important item in their diet, are either producers or accumulators of vitamin B_{12}-active substances (Provasoli and Pintner 1953).

In this investigation the vitamin-B_{12} activity of mullet and shark sera were measured using microbiological assay procedures. An attempt was made to account for the higher vitamin-B_{12} activity of mullet serum by examining the intestinal bacteria of mullet for their ability to produce vitamin B_{12}-active compounds.

METHODS

The blood samples of 10 mullet and 6 sharks (Table 1) were obtained by cardiac puncture with a glass capillary pipet. In

order to avoid contamination by body fluids, only one puncture was made per fish. Approximately 2 to 5 ml of blood from each fish was transferred into test tubes and allowed to clot undisturbed at room temperature (25°C ± 2.0°C) for several hours. The extruded sera were centrifuged at 8,000 rpm for 5 min at 4°C. Samples were either assayed immediately or stored at −5°C for 8 days prior to use.

For comparative purposes 7 serum samples from man were assayed.

Bacteria were isolated from the intestinal contents of mullet using routine bacteriological techniques. The composition of the medium used for isolation and maintenance of stock cultures has been described previously (Starr and Jones 1957).

The serum samples and bacterial isolates were assayed for vitamin-B_{12} activity using *Euglena gracilis, z* strain, and *Escherichia coli* 113–3. Cultures of these assay microorganisms were obtained through the courtesy of Dr. L. Provasoli and Dr. B. D. Davis, respectively. Except for the dilutions of the serum samples which were made with distilled water, the assays were conducted as described earlier (Starr *et al.* 1957).

RESULTS

The relative vitamin-B_{12} activities of mullet, shark, and human sera are listed in Table 1. The activity of mullet sera ranged from 4.6 to 18.4 mμg/ml (av. 10.7), shark sera ranged from 0 to 2.8 mμg/ml (av. 0.84), and human sera ranged from 0.54 to 2.06 mμg/ml (av. 1.15).

TABLE 1. *Relative vitamin-B_{12} activity (mμg/ml) of sera samples as assayed with* Euglena gracilis, *z strain*

Specimen number	Mullet*	Human	Shark	
1	4.6	0.54	2.81	*Aprionodon isodon*
2	13.4	1.07	1.39	" "
3	13.5	0.73	0	*Scoliodon terrae-novae*
4	6.6	1.20	0.20	" "
5	17.0	2.06	0	*Sphyrna diplana*
6	7.6	1.12	0.63	" *tiburo*
7	18.4	1.32		
8	7.8			
9	7.3			
10	10.7			

* All mullet were *Mugil cephalus*.

TABLE 2. *Vitamin B_{12}-production in mμg/ml by bacterial cultures isolated from mullet intestinal contents as assayed with* Escherichia coli *113-3 and* Euglena gracilis, z *strain*

Culture No.	Aliquots of the same culture assayed by	
	E. coli	*Euglena*
233	5.0	1.9
238	1.0	0 (0)*
249	1.3	0 (0.10)
250	0	0 (0)
255	0	0
259	1.1	0
260	0.4	0
266	1.4	0 (11.9)

* The cell residues of mass cultures numbered 238, 249, 250, and 266 were concentrated by centrifugation and assayed. Their corresponding supernatants had no activity for *Euglena*.

Eight bacterial isolates obtained from mullet intestinal contents were grown in a vitamin B_{12}-deficient medium (Starr *et al.* 1957). Aliquots of the same culture were tested with *E. coli* and *Euglena*. Results showed that 6 cultures had activity for *E. coli* and 1 culture had activity for *Euglena*. However, in experiments designed to determine if vitamin B_{12}-active substances were released into the medium or confined to the bacterial cells, we found that concentrates of the cells of 2 out of 4 supposedly negative vitamin-B_{12} producers had positive activity for *Euglena* (Table 2).

DISCUSSION

The specificity of the available microbiological assay organisms for vitamin B_{12}-active substances is discussed in Ford and Hutner (1955). Briefly, *Euglena* is less specific than *Ochromonas* as it responds to pseudovitamin B_{12} and to factors A, C_1, and C_2, but it is more specific than *E. coli* and *Lactobacillus* (Coates and Ford 1955).

The assay techniques incorporating the *Euglena z* strain were developed by Hutner *et al.* (1956). Their procedures eliminated the excessive alkalinization that caused precipitation of serum proteins during growth of *Euglena*. These investigators stated that the mean normal concentration of human sera is about 0.360 mμg/ml. Mollin and Baker (1955) stated that the serum B_{12} concentrations of normal subjects ranged

from 0.100 to 0.900 mμg/ml. Except for one serum which had an activity of 2.06 mμg/ml, we found a range of 0.54 to 1.32 mμg/ml which is in general agreement with the above values.

The activity of serum samples from mullet and shark ranged from 4.6 to 18.4 mμg/ml and 0 to 2.81 mμg/ml, respectively. The higher values for mullet serum compared to shark serum may reflect significant physiological differences between these subclasses of fish and possibly different feeding habits. Although the information in Table 1 is not conclusive, it suggests that the vitamin-B_{12} activity of shark serum differs between genera and species.

Why or how the mullet accumulates such significantly high and variable quantities of vitamin B_{12} in its serum is unknown. This vitamin does play an important role in the metabolism of animals (Arnstein 1955) and microorganisms (Lascelles and Cross 1955). Although the bacterial flora of mullet intestinal contents may not represent a resident population, the relative percentage of vitamin-B_{12} producers in mullet intestine is about the same as that found in marine muds and waters (Starr *et al.* 1957) and in association with seaweeds (Ericson and Lewis 1953). Mullet may obtain vitamin B_{12} either directly from their food or from their intestinal flora or from both sources.

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